Review Article

In Vitro Compression Model in Orthodontics: A Review of Literature

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Abstract

Background: In vitro models provide a controlled and ethically acceptable approach to investigating orthodontic procedures, materials, and biological responses without involving human or animal subjects. They are widely used to study cellular responses, biomechanical properties, friction, bond strength, and material biocompatibility. **Objective:** To review the applications of in vitro models in orthodontic research, with particular emphasis on their potential use in investigating cellular senescence in response to simulated orthodontic forces. **Materials and methods:** This review examines the applications of in vitro models in orthodontic research, including cell culture systems, mechanical testing, friction studies, bond strength assessment, biomechanical analysis, wear testing, and biocompatibility evaluation. Particular attention is given to cellular models involving oral tissue-derived cells, such as fibroblasts, osteoblasts, and periodontal ligament cells, and their responses to simulated orthodontic forces. **Results:** In vitro models have facilitated the investigation of cellular and biomechanical responses to orthodontic forces and materials. Previous cellular studies have identified changes in cellular markers following the application of simulated orthodontic forces. However, the application of in vitro models specifically to investigate cellular senescence under simulated orthodontic loading remains an area requiring further investigation. **Conclusion:** In vitro models offer a valuable platform for investigating biological responses to orthodontic forces under controlled conditions while minimising ethical concerns associated with human and animal studies. Further research is warranted to establish in vitro models for investigating orthodontically induced cellular senescence.

Keywords: Cellular Senescence, In vitro models, Orthodontics

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Introduction

In vitro models are laboratory-based research or studies that are carried out without using living things like people or animals to imitate and comprehend various orthodontic procedures and treatments. These models eliminate the ethical and practical constraints associated with using human subjects in research studies of specific orthodontic treatment components under controlled conditions. Our grasp of orthodontic principles, materials, and techniques is greatly improved by in vitro models. In vitro models can be used for cell culture models, mechanical testing, friction studies, bond strength testing, biomechanical analysis, wear testing and material biocompatibility. Cell culture models are most used as researchers can cultivate many cell types from oral tissues, including fibroblasts, osteoblasts, and cells from the periodontal ligament. Investigating cellular reactions to orthodontic stresses, materials, and therapies can be done using these cell cultures.

Literature review

An overview of in vitro compression models in dentistry

In vitro compression models in orthodontics research can be broadly categorized into two main types: simulated tooth movement systems and craniofacial simulation models. One commonly used in vitro compression model in orthodontics is the simulated tooth movement system. This system typically involves a tooth, or a segment of the dental arch mounted in a device that can apply controlled compressive forces. The resulting tooth movement or other responses, such as changes in bone density or periodontal ligament behaviour, can then be measured and analysed.[1] These models provide insights into the complex interplay between applied forces, tooth movement, and surrounding tissues, which is crucial for optimizing orthodontic treatment planning and outcomes.[2]

Another type of in vitro compression model used in orthodontics is the craniofacial simulation model.[3] These models replicate the biomechanical properties of the craniofacial complex, including the bones, cartilage, and soft tissues.[3] By applying compressive forces to these models, researchers can study the resulting deformations, stresses, and strains within the craniofacial structures. This information can be used to understand the biomechanical response of the face to various orthodontic interventions, such as the use of functional appliances or the application of forces during orthognathic surgery.[4]

The simulated tooth movement system is a widely used in vitro compression model in orthodontics. These models typically involve a single tooth or a segment of the dental arch mounted in a device that can apply controlled compressive forces. By analysing the resulting tooth movement or changes in the surrounding tissues, researchers can gain insights into the biomechanical and biological responses to orthodontic forces.[5,6] These models provide a highly controlled and reproducible environment to study the intricate interplay between the applied forces, tooth movement, and the various components of the periodontium, including the alveolar bone, periodontal ligament, and gingiva.[7]

In contrast, craniofacial simulation models replicate the biomechanical properties of the entire craniofacial complex, including the bones, cartilage, and soft tissues.[8] By applying compressive forces to these models, researchers can investigate the resulting deformations, stresses, and strains within the craniofacial structure.[8] This information is crucial for understanding the biomechanical response of the face to various orthodontic interventions, such as the use of functional appliances or orthognathic surgery.

While both simulated tooth movement systems and craniofacial simulation models offer valuable insights, they differ in their scope and focus. Simulated tooth movement systems primarily concentrate on the local biomechanical and biological responses of

the periodontium, whereas craniofacial simulation models provide a more comprehensive understanding of the overall craniofacial response to applied forces. The choice of in vitro compression model depends on the specific research questions and the level of detail required in the investigation.

In vitro models utilized in orthodontics research

In vitro models made from cell cultures are crucial for investigating different types of tooth movement, orthodontic mechanics and associated processes. In order to study the biological reactions to orthodontic stresses, materials, and treatments, these models entail cultivating cells originating from oral tissues, such as periodontal ligament cells, osteoblasts, and osteoclasts. The key function that periodontal ligament cells play in the transmission of pressures from orthodontic appliances to the tooth and surrounding bone makes them essential for orthodontic studies.[9,10] It is possible to apply mechanical stresses to cultured cells that mimic orthodontic settings. By simulating the forces applied by braces, wires, and aligners, researchers examine how cells react to compression, tension, and shear stress.[2] We can better understand inflammation and tissue remodelling by using in vitro models that investigate how cells respond to mechanical loading by secreting cytokines and growth factors.[11] In vitro compression models have also been employed to investigate the effects of orthodontic forces on the periodontal tissues.[12] These models can simulate the application of compressive forces on the periodontal ligament and alveolar bone, allowing researchers to study the resulting cellular and tissue-level changes.[12] This information is crucial for understanding the biological mechanisms underlying tooth movement and the potential risks associated with excessive or uncontrolled orthodontic forces,[13] such as root resorption or loss of supporting bone.

The clinical significance of in vitro compression models in orthodontics research is multifaceted. These models provide a controlled and reproducible environment to investigate the biomechanical and biological responses of the periodontium during orthodontic tooth movement, which is not easily achievable in clinical settings.[14] The insights gained from these models can inform the development of more effective and safe orthodontic treatment protocols, leading to improved patient outcomes and reduced risk of adverse effects, such as root resorption or alveolar bone loss.[15]

Furthermore, in vitro compression models can be used to evaluate the performance and design of orthodontic appliances, such as braces, aligners, or functional appliances. By simulating the compressive forces experienced by these appliances during use, researchers can assess their biomechanical characteristics, optimize their designs, and ensure their clinical effectiveness and safety.[14-16]

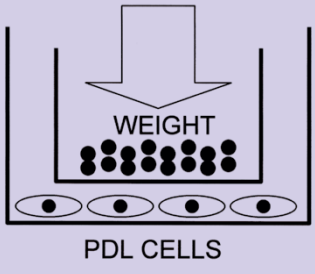
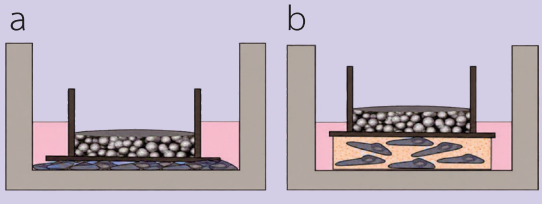
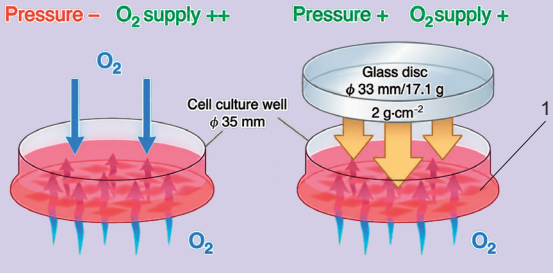
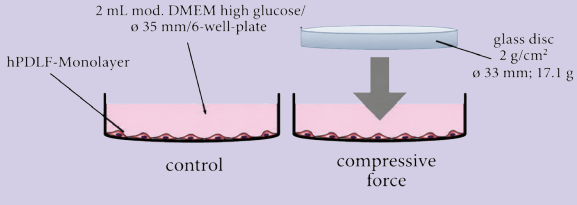
In summary, in vitro compression models have become an essential tool in orthodontics research, providing valuable insights into the biomechanical and biological responses of the periodontium to applied forces. These models enable a deeper understanding of the complex processes involved in orthodontic tooth movement, leading to the development of more efficient and patient-centered treatments.[15,16]

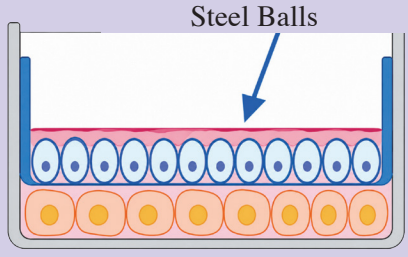
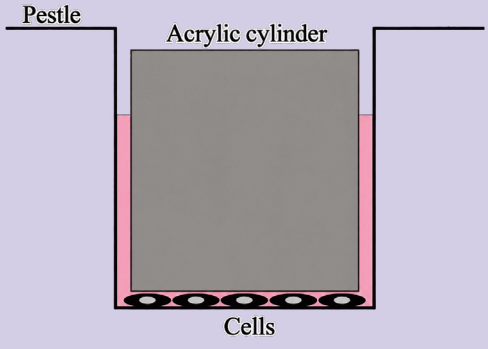
In vitro model studying cellular changes due to simulated orthodontic forces

Due to different patients and treatment approaches, the exact magnitude of orthodontic forces on periodontal cells in vivo can vary.[17] The consensus of achieving orthodontic tooth movement is applying force without detrimental tissue damage. Despite this, a well-established in vitro model of the early phase of orthodontic tooth movement compression side in HPDLF is already capable of inducing a reactive synthesis of proinflammatory cytokines and remodelling molecules.[1] A model which consisted of glass cylinders exerting a static compressive force of 2g/cm^2 on

a cell monolayer, was used to observe the process of HPDLF restructurement.[12] It is hypothesised that HPDLF generally die due to compression and primarily contribute to periodontal tissue organisation during orthodontic tooth movement via the inflammatory response.[18,19] A study which used an in vitro model HPDLF restore homeostasis by preserving cellular structures under static compression via a slower cell cycle and a lower division rate.[12] According to this study, the reduction of proliferation is a critical part of the re-organisation process of HPDLFs under compressive force, in addition to cellular inflammation and cell death.[12]

Table 1 Comparing different in vitro models simulating orthodontic compression forces.

Year	Research Title & Author	Research Objective	In Vitro Model Components
2002	Periodontal Ligament Cells Under Mechanical Stress Induce Osteoclastogenesis by Receptor Activator of Nuclear Factor B Ligand Up-Regulation via Prostaglandin E2 Synthesis by Kanzaki, et al.[1]	To study how mechanical stress affects the osteoclastogenesis-supporting activity of PDL cells	
2018	In Vitro Weight-Loaded Cell Models for Understanding Mechanodependent Molecular Pathways Involved in Orthodontic Tooth Movement: A Systematic Review by Janjic, et al.[18]	Systematic review to identify all studies in the field of dentistry with focus on mechanobiology using in vitro loading models applying uniaxial static compressive force	
2019	The role of mechanotransduction versus hypoxia during simulated orthodontic compressive strain—an in vitro study of human periodontal ligament fibroblasts by Ullrich, et al.[19]	To investigate expression patterns of genes and proteins that were previously identified to be associated with orthodontic tooth movement after compressive force application in the presence of a normal or reduced oxygen supply	
2021	In Vitro Compression Model for Orthodontic Tooth Movement Modulates Human Periodontal Ligament Fibroblast Proliferation, Apoptosis and Cell Cycle by Brockhaus, et al.[11]	To study morphology of hPDLF changes towards more unstructured, unsorted filaments under mechanical compression	

Year	Research Title & Author	Research Objective	In Vitro Model Components
2023	CD97 inhibits osteoclast differentiation via Rap1a/ERK pathway under compression by Wang, et al.[20]	To investigate the relationship between CD97 expression and osteoclast differentiation using models of OTM and static compression application	
2024	Effects of continuous and released compressive force on osteoclastogenesis in vitro by Changkhaokam, et al.[21]	To quantify the fusion of mononuclear RAW 264.7 cells (a murine osteoclastic-like cell line) into multinucleated osteoclasts under a hydrostatic pressure-generated mechanical compression-the new model of various magnitudes and durations.	

In vitro compression model and cellular senescence

In vitro compression models have emerged as valuable tools to study the impact of orthodontic forces on cellular senescence. These models allow researchers to simulate the compressive stresses experienced by periodontal tissues during orthodontic treatment and investigate the resulting cellular and molecular changes.[22,23]

By applying controlled compressive forces to simulated tooth movement systems or craniofacial simulation models, researchers can examine markers of cellular senescence, such as increased expression of cell cycle regulators like p16 and p21, telomere shortening, and elevated senescence-associated beta-galactosidase activity.[24,25] Examining these senescence-related markers in the periodontal ligament and alveolar bone cells can offer valuable insights into how orthodontic forces may lead to premature aging and impairment of these crucial cell populations. This opens new research avenues to help understand the biological mechanisms behind senescence in orthodontics and develop strategies to mitigate potential adverse effects.

In vitro model predictability and measured outcome

In vitro models can be quite predictable, particularly when experimental settings are carefully regulated and standardized. Researchers can controllably manipulate specific variables, such as cell types, drug concentrations, and environmental parameters, using in vitro models. Under these specific conditions, this control can lead to predictable responses. In vitro models are valuable for examining the underlying mechanisms of biological processes. Predictions about how cells will react to particular interventions or stimuli can be more precise when the pathways involved are well-understood. However, in vitro models may be hindered by a lack of the complexity found in living organisms. They fall short of accurately capturing the intricate relationships between the many organs, cell types, and physiological systems present in vivo.[25] Additionally, the numerous connections and feedback loops in real organisms can produce results that may not be straightforward to anticipate based on individual in vitro studies. Studies of cells, tissues, or organisms in a controlled

laboratory setting, separate from a living organism, are frequently conducted using in vitro models.[20] Imaging is essential to these models, as it enables the visualization and analysis of various cellular and molecular processes.[26] Specific molecules or cell structures are marked with fluorescent dyes or tags to facilitate this analysis.[27]

The visualisation of dynamic processes and particular biological components is made possible by fluorescence microscopy. Fluorescence microscopy is a powerful imaging method that makes use of fluorescence to see particular chemicals, structures, or activities inside of cells and tissues.[28] Target molecules are marked with fluorescent proteins or dyes to enable non-invasive and real-time observation of their distribution, localization, and interactions.[29] When used with in vitro models, fluorescence microscopy offers useful insights into the dynamic features of biological processes. The ability to directly witness previously invisible molecular interactions and events has revolutionised cell biology. For researching biological reactions to stress in in vitro models, fluorescence microscopy is an invaluable tool.[26] In this context, the term "stress" can be used to describe a variety of physiological and environmental difficulties that cells may encounter, including oxidative stress, heat shock, nutritional shortage, and others. Fluorescence microscopy techniques enable researchers to see and examine how cells respond to stimuli, determine the effect on cellular constituents, and investigate the underlying mechanisms.[29] This method also produces a set of pictures and information that helps scientists comprehend in detail how cells function, how chemicals interact, and how biological molecules behave in an in vitro model.[30] Although in vitro models provide insights into cellular behaviour, it's crucial to recognise that before conclusions from cell culture studies can be directly applied to medical care, they must first be verified through animal models and clinical trials.

Discussion

The move towards emerging in vitro models, particularly 3D tissue cultures, represents a significant step forward in orthodontic research. These models strive to more accurately replicate the complex physiological conditions and interactions present in the in vivo environment, bridging the gap between traditional in vitro methods and the inherent complexity of animal models. In vitro models offer a more ethically sound alternative to human and animal testing. This is a crucial consideration, especially as research delves deeper into the effects of orthodontic forces at the cellular level.

Conclusion

Emerging in vitro models, including 3D tissue cultures, aim to bridge the gap between conventional in vitro models and the complexity of animal models. These new technologies have the potential to more accurately mimic physiological conditions and interactions. To date, there is no established in vitro model examining cellular senescence under simulated orthodontic forces. Previous cellular studies have identified new markers when applying simulated forces. In vitro models remain the best option for cellular studies, as they are more ethically safe compared to human and animal testing.

Author contributions

Naadira Jamaluddin: Conceptualization, Investigation, Data curation, Writing and Original Draft; **Nik Mukhriz Nik Mustapha:** Supervision; **Aida Nur Ashikin Abd Rahman:** Supervision, Project administration; **Luay Thanoon Younis:** Supervision; **Syatirah Najmi Abdullah:** Validation and Review of the scientific and technical content; **Fara Fariza Zahar:** Methodology and Validation.

Disclosure Statement

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AI-Assisted Writing Declaration

During the preparation of this manuscript, the authors used ChatGPT (OpenAI, GTP-5.5) solely for language editing, including grammar, punctuation, and clarity of expression. The authors reviewed and approved all changes and take full responsibility for the content and accuracy of the manuscript.

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References

1. Kanzaki H, Chiba M, Shimizu Y, Mitani H. Periodontal ligament cells under mechanical stress induce osteoclastogenesis by receptor activator of nuclear factor kappaB ligand up-regulation via prostaglandin E2 synthesis. J Bone Miner Res 2002;17(2):210-20.
2. Sun C, Janjic Rankovic M, Folwaczny M, Stocker T, Otto S, Wichelhaus A, et al. Effect of different parameters of in vitro static tensile strain on human periodontal ligament cells simulating the tension side of orthodontic tooth movement. Int J Mol Sci 2022;23(3):1525.
3. Zong C, Van Dessel J, Vande Velde G, Willems G, Cadenas de Llano-Pérula M. Dynamic changes in tooth displacement and bone morphometry induced by orthodontic force. Sci Rep 2022;12(1):13672.
4. Pilon JJ, Kuijpers-Jagtman AM, Maltha JC. Magnitude of orthodontic forces and rate of bodily tooth movement. An experimental study. Am J Orthod Dentofacial Orthop 1996;110(1):16-23
5. Jeon HH, Teixeira H, Tsai A. Mechanistic insight into orthodontic tooth movement based on animal studies: a critical review. J Clin Med 2021;10(8):1733.
6. Chen J, Li W, Swain MV, Ali Darendeliler M, Li Q. A periodontal ligament driven remodeling algorithm for orthodontic tooth movement. J Biomech 2014;47(7):1689-95.
7. Meikle MC. The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. Eur J Orthod 2006;28(3):221-40.
8. Yang YQ, Li XT, Rabie AB, Fu MK, Zhang D. Human periodontal ligament cells express osteoblastic phenotypes under intermittent force loading in vitro. Front Biosci 2006;11: 776-81.
9. Feller L, Khammissa RA, Schechter I, Thomadakis G, Fourie J, Lemmer J. Biological events in periodontal ligament and alveolar bone associated with application of orthodontic forces. ScientificWorldJournal 2015;2015:876509.
10. Veith A, Conway D, Lei MG, Eskin SG, McIntire LV, Baker AB. Effects of mechanical forces on cells and tissues. In: Veith D, Conway MG, Lei SG, Eskin LV, McIntire, Baker AB, editor. Biomaterials Science. Royal Society of Chemistry; 2020.p.717
11. Brockhaus J, Craveiro RB, Azraq I, Niederau C, Schröder SK, Weiskirchen R, et al. In vitro compression model for orthodontic tooth movement modulates human periodontal ligament fibroblast proliferation, apoptosis and cell cycle. Biomolecules 2021;11(7):932.
12. Carney KA, Colloc TNE, Kilgariff JK. Management of rarely seen internal tunnelling root resorption associated with a maxillary permanent incisor. Br Dent J 2024;236(12):955-61.
13. Li Y, Zhan Q, Bao M, Yi J, Li Y. Biomechanical and biological responses of periodontium in orthodontic tooth movement: up-date in a new decade. Int J Oral Sci 2021;13(1):20.
14. Heidary Z, Mojra A, Shirazi M, Bazargan M. A novel approach for early evaluation of orthodontic process by a numerical thermomechanical analysis. Int J Numer Method Biomed Eng 2018;34(1).
15. Barone S, Paoli A, Razionale AV, Savignano R, editor. 3D reconstruction of individual tooth shapes by integrating dental CAD templates and patient-specific anatomy. Proceedings of the ASME 2014 International Design Engineering Technical Conferences and Computers and Information in Engineering Conference; 2014; New York, USA. New York: The American Society of Mechanical Engineers (ASME); 2014.
16. Mohamad Kamal NS, Safuan S, Shamsuddin S, Foroozandeh P. Aging of the cells: insight into cellular senescence and detection methods. Eur J Cell Biol 2020;99(6):151108.
17. Weider M, Schröder A, Docheva D, Rodrian G, Enderle I, Seidel CL, et al. A human periodontal ligament fibroblast cell line as a new model to study periodontal stress. Int J Mol Sci 2020;21(21):7961.

18. Janjic M, Docheva D, Trickovic Janjic O, Wichelhaus A, Baumert U. In Vitro Weight-loaded cell models for understanding mechanodependent molecular pathways involved in orthodontic tooth movement: a systematic review. Stem Cells Int 2018;2018:3208285.
19. Ullrich N, Schröder A, Jantsch J, Spanier G, Proff P, Kirschneck C. The role of mechanotransduction versus hypoxia during simulated orthodontic compressive strain-an in vitro study of human periodontal ligament fibroblasts. Int J Oral Sci 2019;11(4):33.
20. Wang W, Wang Q, Sun S, Zhang P, Li Y, Lin W, et al. CD97 inhibits osteoclast differentiation via Rap1a/ERK pathway under compression. Int J Oral Sci 2024;16(1):12.
21. Changkhaokham B, Suamphan S, Jitpukdeebodintra S, Leethanakul C. Effects of continuous and released compressive force on osteoclastogenesis invitro. J Oral Biol Craniofac Res 2024;14(2):164-8.
22. Li M, Zhang C, Yang Y. Effects of mechanical forces on osteogenesis and osteoclastogenesis in human periodontal ligament fibroblasts: a systematic review of in vitro studies. Bone Joint Res. 2019;8(1):19-31.
23. Veronesi F, Contartese D, Di Sarno L, Borsari V, Fini M, Giavaresi G. In vitro models of cell senescence: a systematic review on musculoskeletal tissues and cells. Int J Mol Sci 2023;24(21):15617.
24. Zhou Y, Nishiura A, Morikuni H, Deng W, Tsujibayashi T, Momota Y, et al. RANKL+ senescent cells under mechanical stress: a therapeutic target for orthodontic root resorption using senolytics. Int J Oral Sci 2023;15(1):20.
25. Kaplan M, Kalajzic Z, Choi T, Maleeh I, Ricupero CL, Skelton MN, et al. The role of inhibition of osteocyte apoptosis in mediating orthodontic tooth movement and periodontal remodeling: a pilot study. Prog Orthod 2021;22(1):21.
26. Mohanakumar A, Vijay GL, Vijayaraghavan N, Rajendran RS, Chandran MB, Thulasidharan MU, et al. Morphological alterations, activity, mRNA fold changes, and aging changes before and after orthodontic force application in young and adult human-derived periodontal ligament cells. Eur J Orthod 2021;43(6):690-6.
27. Galvis D, Walsh D, Harries LW, Latorre E, Rankin J. A dynamical systems model for the measurement of cellular senescence. J R Soc Interface 2019;16(159):20190311.
28. Parkinson EK, Prime SS. Oral senescence: from molecular biology to clinical research. Front Dent Med 2022;3:822397.
29. Younis LT, Abu Hassan MI, Taiyeb Ali TB, Bustami TJ. 3D TECA hydrogel reduces cellular senescence and enhances fibroblasts migration in wound healing. Asian J Pharm Sci 2018;13(4):317-25.
30. Paek J, Song JW, Ban E, Morimitsu Y, Osuji CO, Shenoy VB, et al. Soft robotic constrictor for in vitro modeling of dynamic tissue compression. Sci Rep 2021;11(1):16478.